# Metagenome@KIN
# User Manual
# ver2.01

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# I.Outline

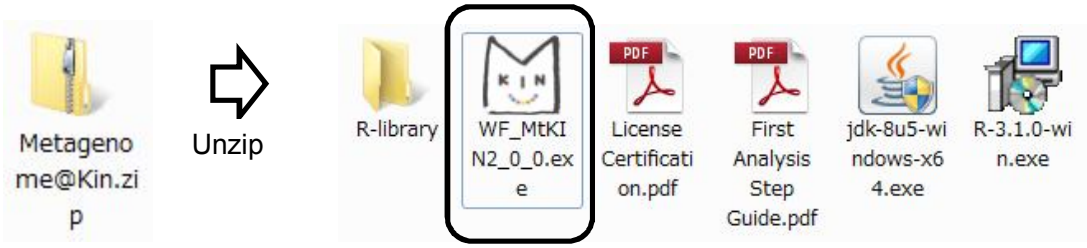
Metagenome@KIN 16S rRNA Analysis Tool provides following results by inputting BLAST result files.

- ✓ Rejected Information (information rejected by filters, containing names of reads and the number of reads)
- ✓ Pie Chart/Sunburst Graph
- ✓ Bar Graph
- ✓ Principal Component Analysis (2D)
- ✓ Principal Component Analysis (3D Animation)
- ✓ Principal Component Analysis (3D Interactive)
- ✓ Hierarchical Clustering
- ✓ Self-organizing Maps(SOM)

# II.Install（Windows）

## Ⅱ－Ⅰ. Install Steps

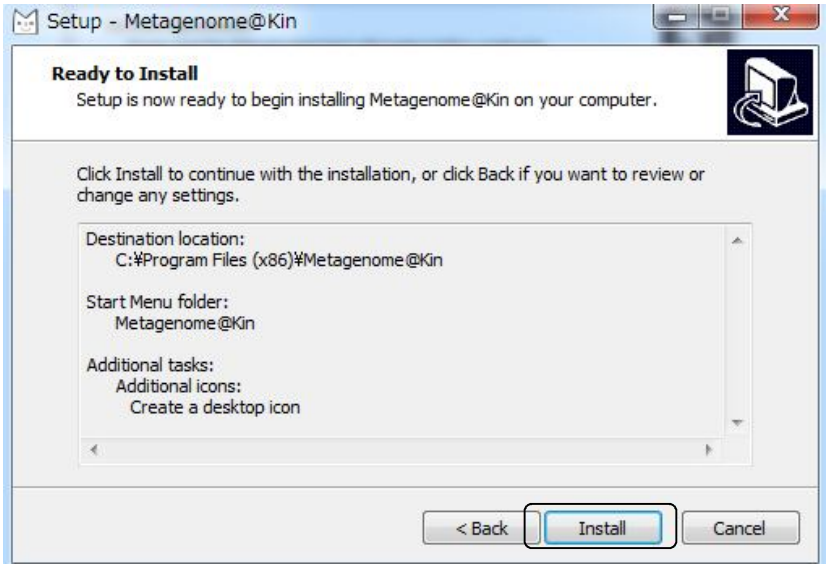
Decompress Metagenome@Kin zip archive you have downloaded and double-click the “WF_MtKIN2_0_0.exe”installer.



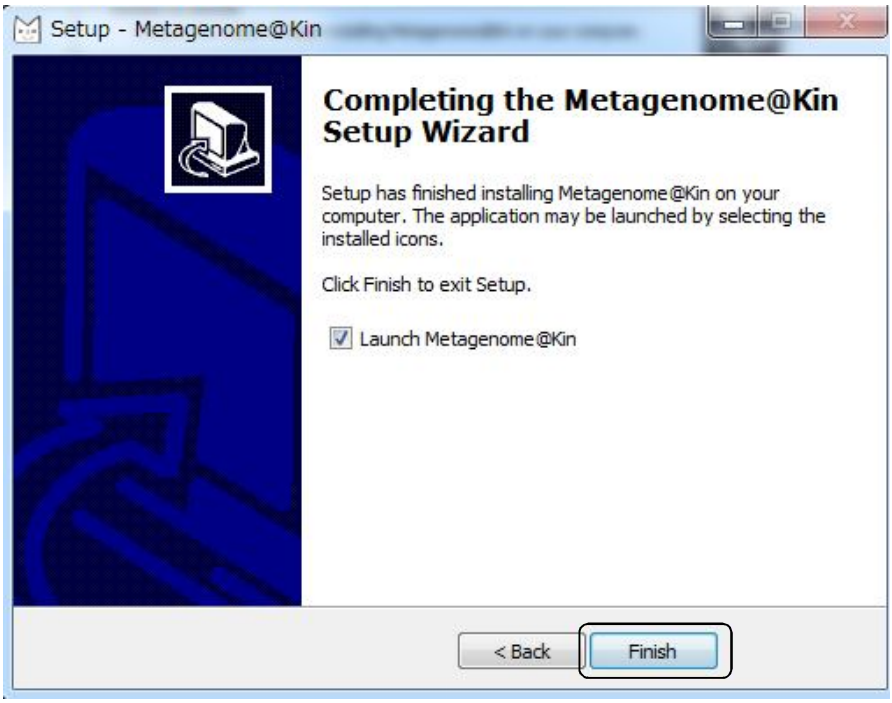

Select “I accept the agreement” and go to “Next >”.

Setup - Metagenome@Kin
License Agreement
Please read the following important information before continuing.
Please read the following License Agreement. You must accept the terms of this agreement before continuing with the installation.
I accept the agreement
I do not accept the agreement
< Back
Next >
Cancel

Decide a directory for the installing destination and a shortcut icon. Then click “Install”.


Setup - Metagenome@Kin
Ready to Install
Setup is now ready to begin installing Metagenome@Kin on your computer.
Click Install to continue with the installation, or click Back if you want to review or change any settings.
Destination location:
C:¥Program Files (x86)¥Metagenome@Kin
Start Menu folder:
Metagenome@Kin
Additional tasks:
Additional icons:
Create a desktop icon
< Back
Install
Cancel

Click “Finish” to finish the installation.

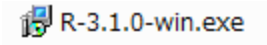

Setup - Metagenome@Kin
Completing the Metagenome@Kin Setup Wizard
Setup has finished installing Metagenome@Kin on your computer. The application may be launched by selecting the installed icons.
Click Finish to exit Setup.
Launch Metagenome@Kin
< Back
Finish

## Ⅱ－Ⅱ. Install of R and R libraries

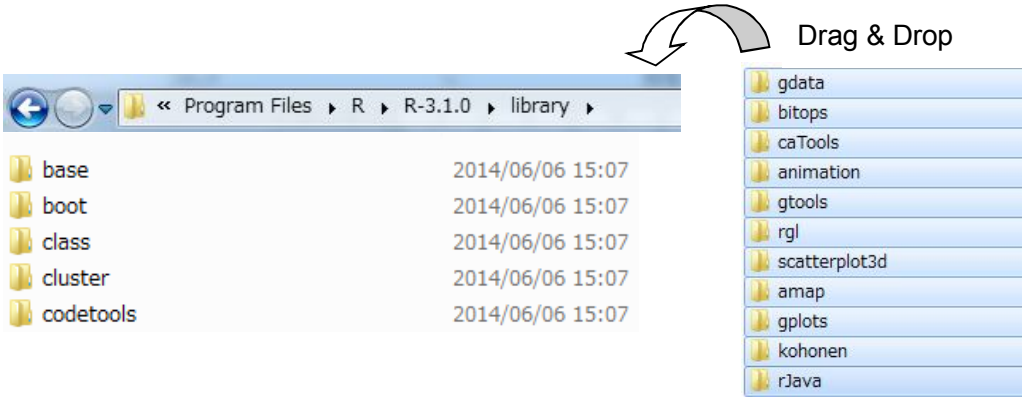
Install R and R libraries.

You have downloaded Metagenome@Kin zip archive contains R installer and R libraries.

Double-click the R installer as below and follow the instruction showing on the window as default. It will be usually installed under the folder “C:¥Program Files¥R¥R-3.1.0”.




After the R installation, transfer all the R libraries to the R library folder (¥R¥R-3.1.0¥library).



## Ⅱ-Ⅲ. Install of Java

Obtain the Java installer from the following link.

Java

http://www.oracle.com/technetwork/java/javase/downloads/index.html

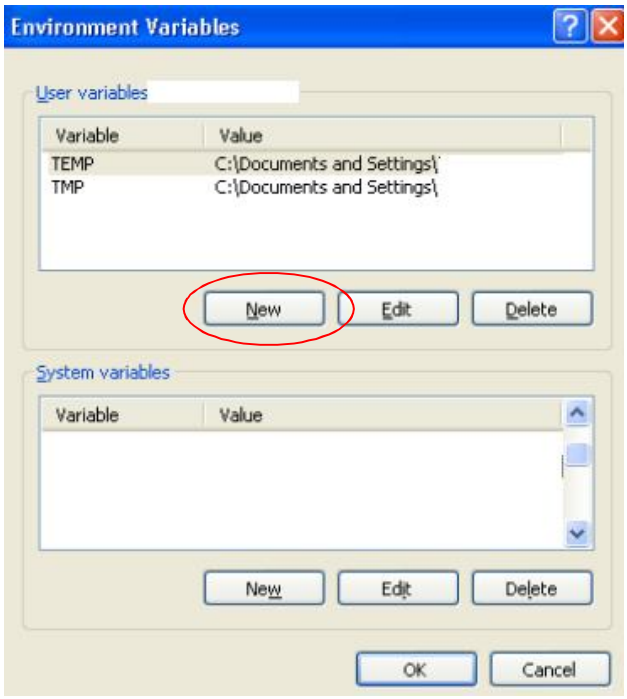
Double-click the Java installer as below and follow the instruction showing on the window as default.

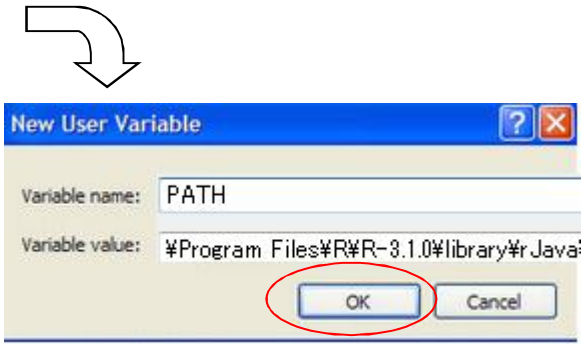
jdk-8u5-windows-x64.exe

## Ⅱ-Ⅳ. PATH Setting

Set up a path of the R library in order to execute R on Metagenome@KIN.

Open "Control Panel", go to System, Advanced system settings, and click the Advance tab and click "Environment Variables". The following window will pop up.




Click "New" and enter "PATH" as a variable name.

Enter the whole path (e.g., C:¥Program Files¥R¥R-3.1.0¥bin¥x64) for R x64 into variable value.

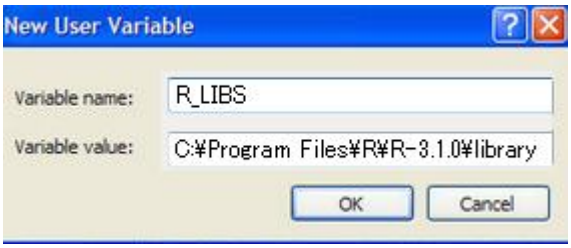
In addition, enter the whole path (e.g., C:¥Program Files¥R¥R-3.1.0¥library¥rJava¥jri¥x64) to x64 under the jri folder of R library (rJava) after putting a semi colon. Example is shown as below.

C:¥Program Files¥R¥R-3.1.0¥bin¥x64;C:¥Program Files¥R¥R-3.1.0¥library¥rJava¥jri¥x64

Set up a path to R library folder.

Click "New" and enter "R_LIBS" as a variable name.

Next enter the whole path (e.g., C:¥Program Files¥R¥R-3.1.0¥library) for R library folder in the variable value.




Click “OK” and finish the Environment Variables setting.

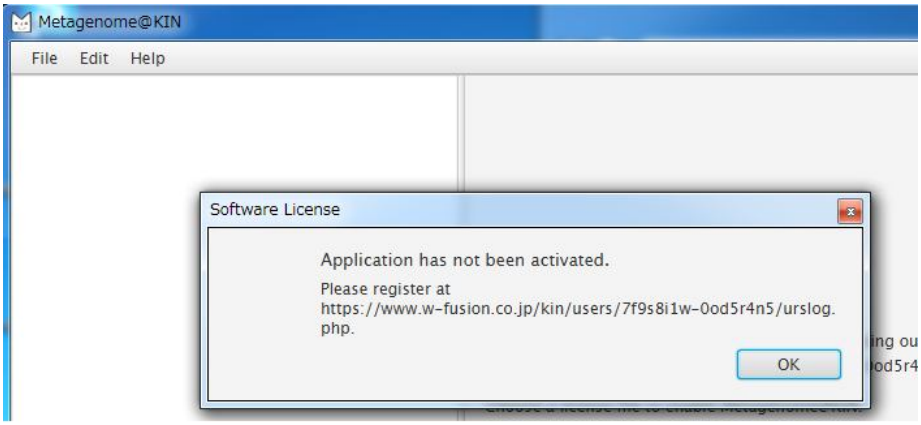
You can start the application created by the shortcut of Metagenome@Kin on the Desktop.

If you have any problems during the install process, please contact our customer support.

E-mail: techsupport@w-fusion.co.jp

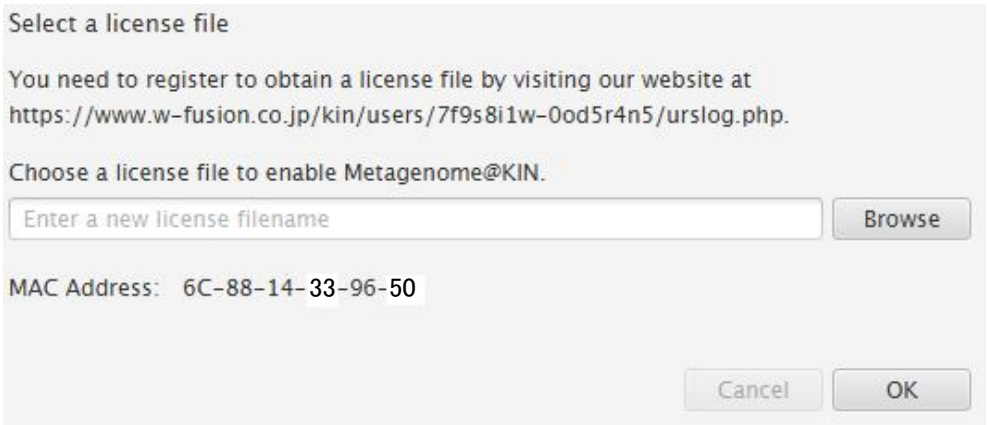
## III.License

Demo license term is two weeks. Following message will pop up when your demo license term has expired.
Open the shown URL on the message for a user authorization.



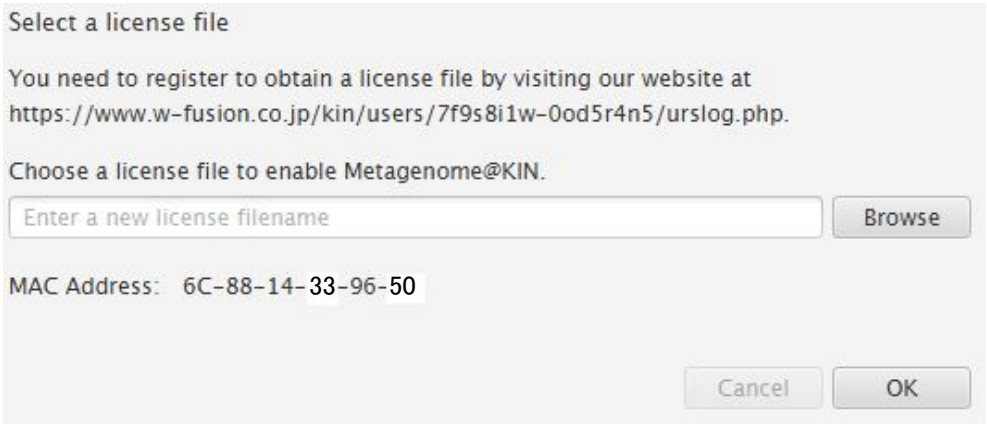

On the license authorization page, enter the followings
User name
Organization name
Email address
and physical address(=MAC address)* of the computer

A license file will be emailed to the entered email address from World Fusion.
The software will be available by selecting KIN license from the file.


Select a license file
You need to register to obtain a license file by visiting our website at
https://www.w-fusion.co.jp/kin/users/7f9s8i1w-0od5r4n5/urslog.php.
Choose a license file to enable Metagenome@KIN.
Enter a new license filename
Browse
MAC Address: 6C-88-14-33-96-50
Cancel
OK

* How to obtain physical address(=MAC address)

Method 1: MAC address of the installed PC will be displayed on the KIN license authorization window. Type the numbers in the entry box on the license authorization web page.

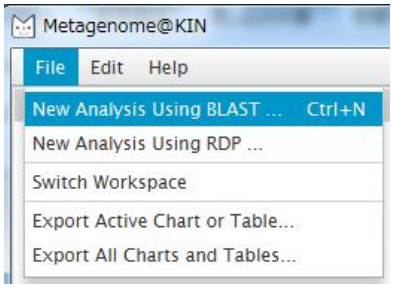

Select a license file
You need to register to obtain a license file by visiting our website at
https://www.w-fusion.co.jp/kin/users/7f9s8i1w-0od5r4n5/urslog.php.
Choose a license file to enable Metagenome@KIN.
Enter a new license filename
Browse
MAC Address: 6C-88-14-33-96-50
Cancel
OK

Method 2: (For Windows) open Command prompt and enter “getmac”. Then MAC address will be shown.

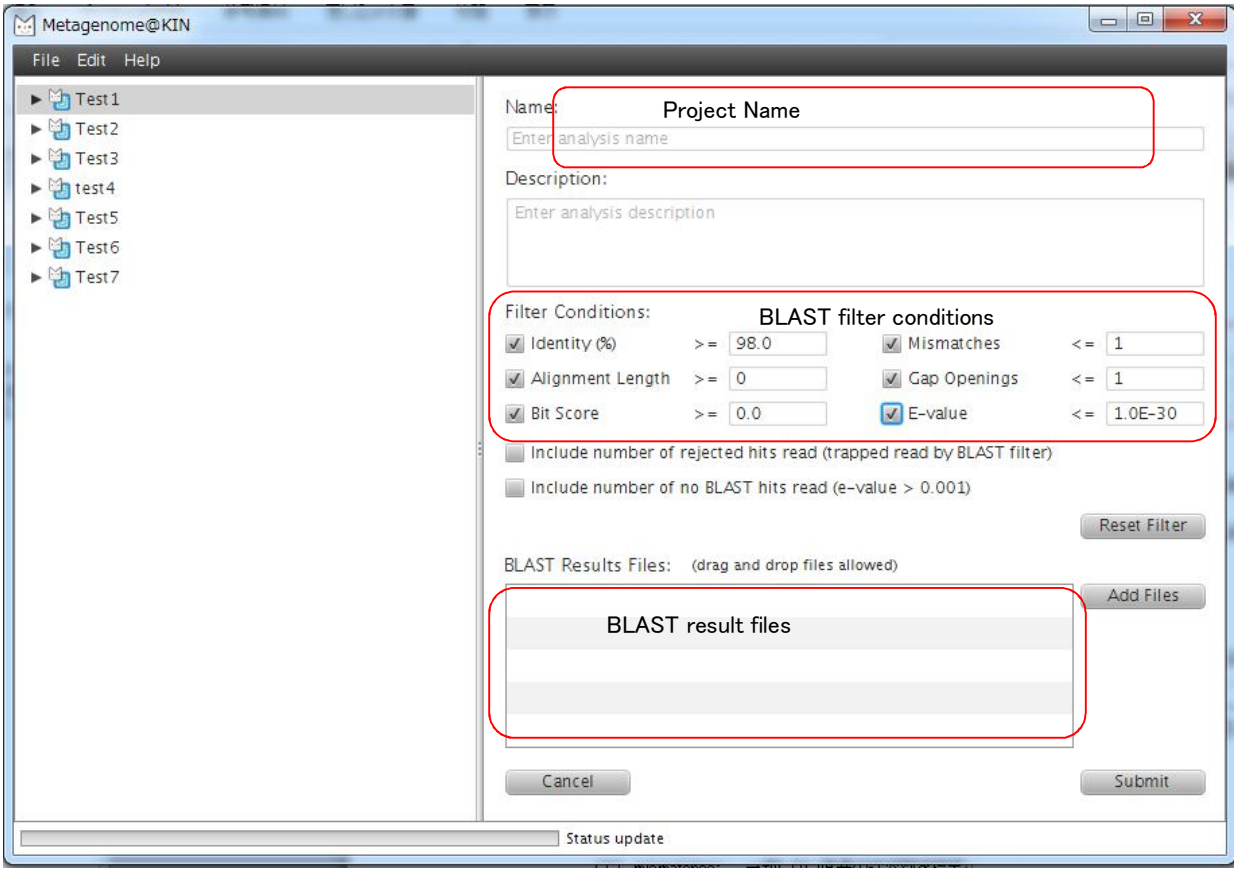
# IV.Analysis Execution from BLAST result

## Ⅳ－Ⅰ. Analysis Execution

Select “New Analysis using BLAST” from File tab. The following analysis start menu will be displayed.




Enter analysis project name (Required)




You can alter the filter conditions for BLAST results. Once you click “Reset Filter”, it goes back to the default setting.

Identity (%): the lower limit of the sequence identity　alignmentlength: the lower limit of the alignment length

bitscore: the lower limit of twice the length of homologous local alignement

mismatches: the number of non-matched bases

gapopenings: the number of base gaps　e-value: the upper limit of the e-value

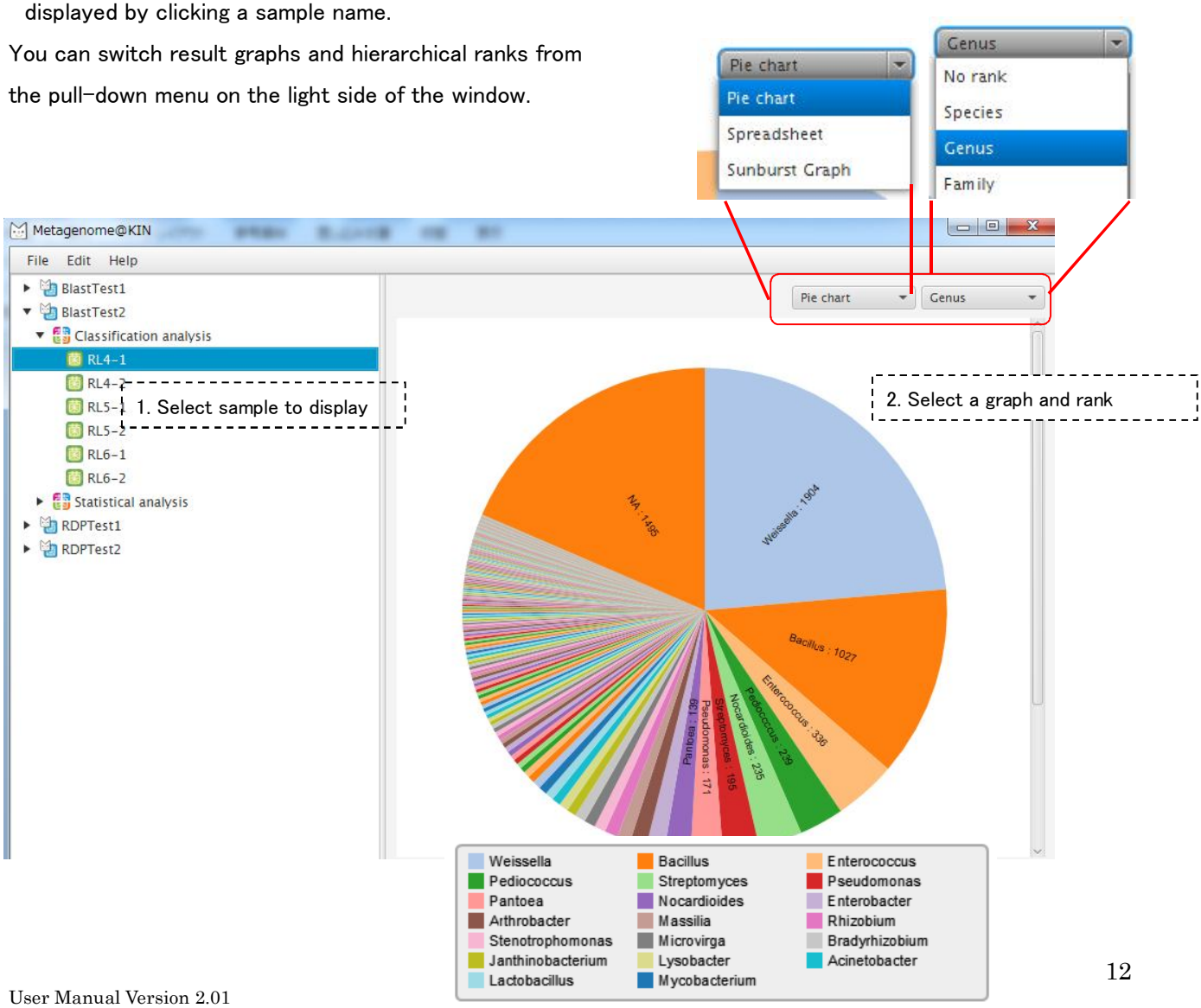
After setting the filter condition, drag and drop BLAST result files on the input area. Multiple files can be added. Click “Submit” to start analysis.

Once the analysis starts, each entered BLAST file name will be displayed as its sample name in a folder under the entered project name with Classification analysis folder. Analysis process will be completed once "Complete the number of entered samples of the number of entered samples" as shown below.

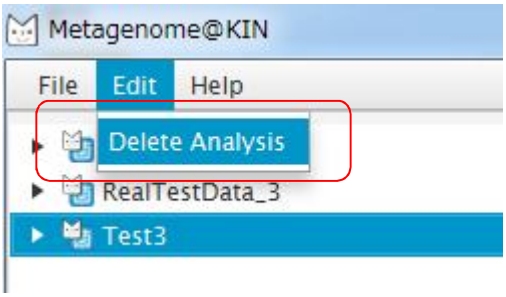
## Ⅳ－Ⅱ. Analysis Result Display

On the left side of the analysis window, Pie chart/Sunburst graph/spreadsheet as analysis results will be displayed by clicking a sample name.

You can switch result graphs and hierarchical ranks from the pull-down menu on the light side of the window.



You can delete each analysis project result by selecting the project and clicking Delete Analysis on Edit tab.

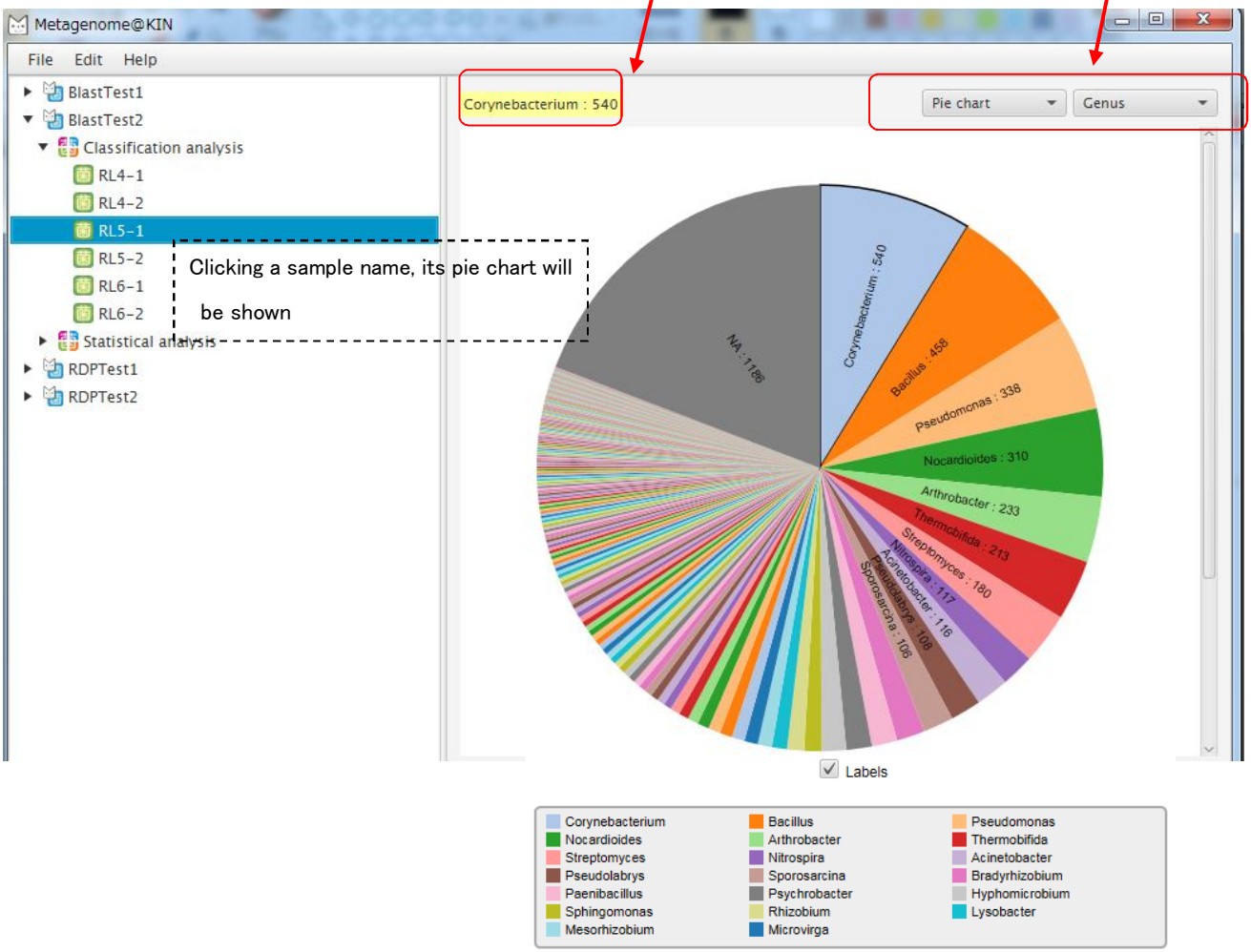


## V.Pie Chart Details

In the Pie chart, the top 20 (the number of hit reads) of microbes will be listed below the graph

Selected microbe name and the number of hit reads

Graph display and rank



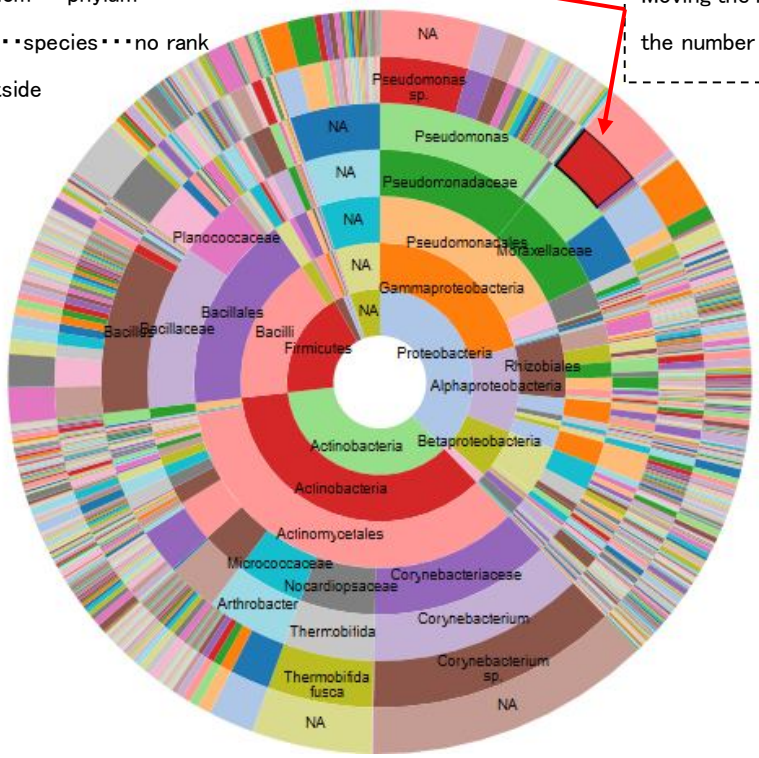
## VI.Sunburst Graph Details

Species : Acinetobacter sp. : 75

Sunburst Graph | Family

Sunburst graph places, superkingdom･･･phylum･･･class･･･order･･･family･･･genus･･･species･･･no rank in order from its center to the outside

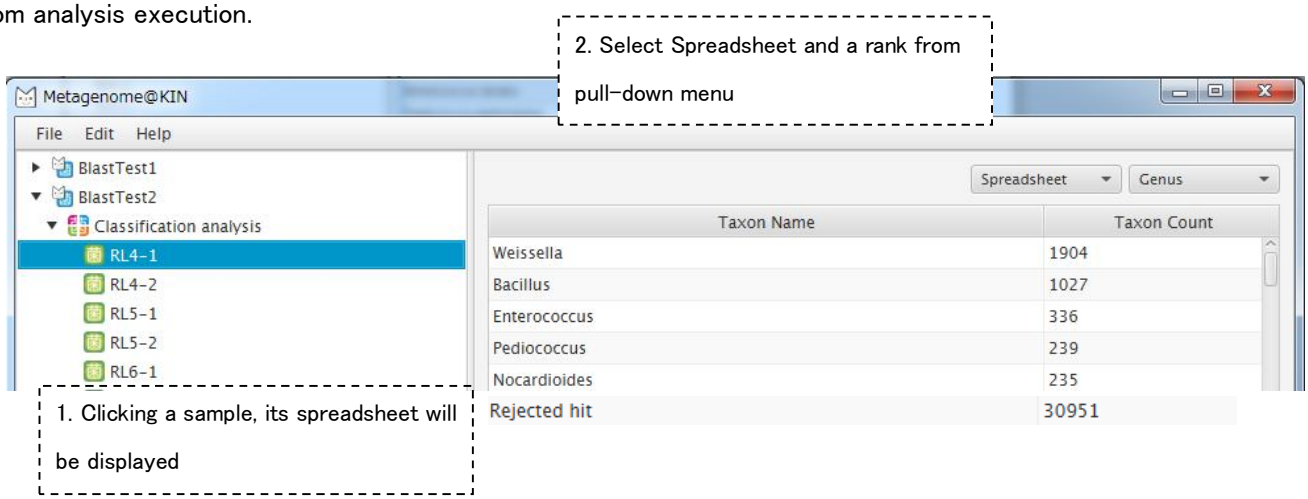
Moving the mouse pointer, the microbe and the number of hit reads will be displayed.



# VII.Analysis Result Outputs

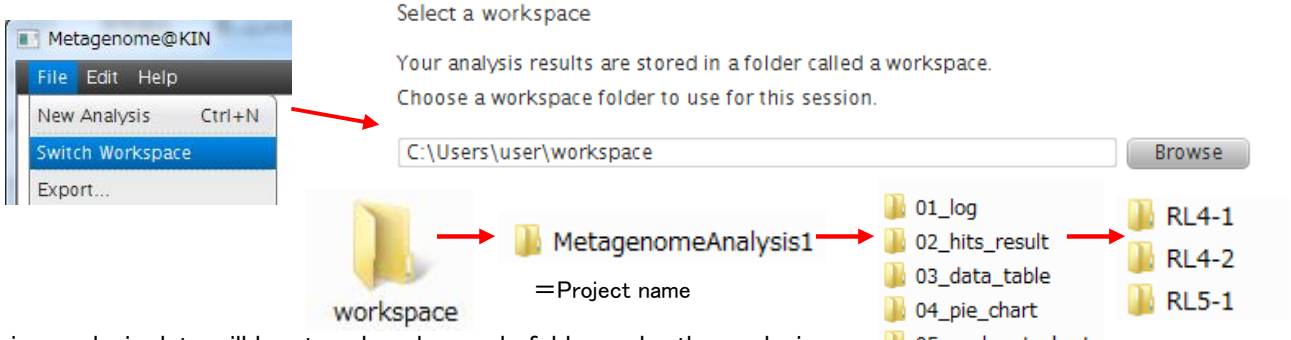
## Ⅶ－Ⅰ. Spreadsheet Display

Spreadsheet describes microbial frequency for each sample. The frequency (= the number of read) is hierarchically classified. “Rejected reads” indicates the excluded number of reads through filter conditions from analysis execution.




## Ⅶ－Ⅱ. Save Location of Analysis Data Files

Metagenome@KIN analysis data automatically create a folder (sample name) in the project name folder to store results as shown below (File –> Switch Workspace). The saved location (directory) can be modified.




Following analysis data will be stored each sample folder under the analysis Menu folder.

CSV file: Spreadsheet data for each rank. Pie chart is created from this data.

HTML file: Sunburst and Pie chart graph data, your need FireFox to see the result.

accepted.csv in 02_hits_result folder: Data of reads in which strain each read belongs to through metagenome analysis.

rejected.csv in 02_hits_result folder: Excluded reads from analysis based on filter conditions.

no_blast.csv in 02_hits_result folder: Excluded reads from analysis based on BLAST setting.

## Ⅶ-Ⅲ. Outputs of Analysis Data

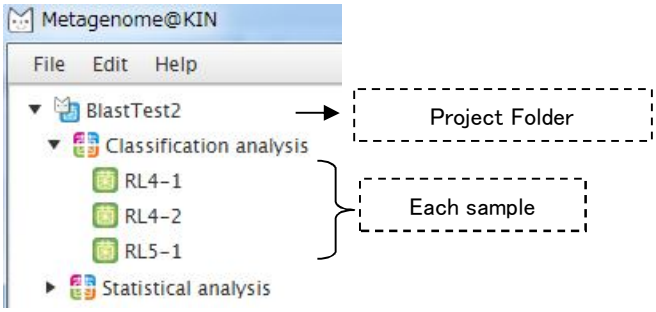
Graph data such as pie chart and sunburst graph can be exported by selecting File –> Export Active Chart or Table from menu. The graph image will be a HTML document and table will be a CSV format. Those data are available under FireFox and Excel.

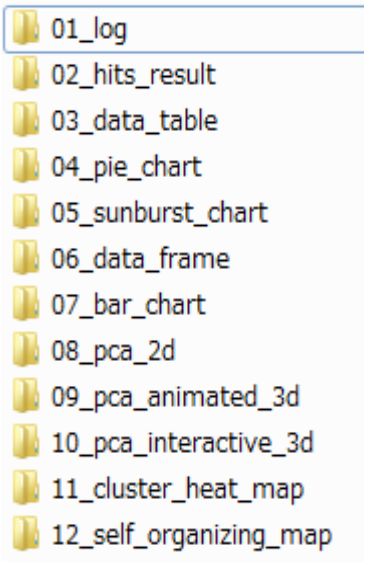
*In the case of the large number of microbes, it is possible that pie chart and sunburst graph may not be displayed on the application. In this case, by exporting HTML results, you can see the outputs on the web browser instead (File –> Export Active Chart or Table). FireFox will be required to see the exported image.

In order to export all data in a project, select File –> Export All Charts and Tables.

Once your select a stored location, all graphs (in HTML), tables (in CSV), and cluster analysis results will be exported.

Following information will be exported.




----- analysis log file

-----Assigned bacteria name table for each read

-----Frequency analysis result table for each ranks

-----pie chart image and pie graph source data(in CSV)

-----sunburst graph image (in html)

-----Frequency analysis result table throw all samples

-----bar graph image(in html) and bar graph source data(in CSV)

-----2D PCA analysis result image

-----3D PCA analysis result image(auto frame-by-frame)

-----3D PCA analysis result image(manual frame by frame)

-----hierarchical clustering result image

-----SOM analysis result image

Contents of bar_chart folder:

Bar graph image data for each rank (FireFox is required)

Bar graph source data for each rank (microbial frequency information of reads for each analysis sample)

Contents of pie_chart folder:

Pie chart graph source data for each rank （microbial frequency information of reads for each analysis sample）

Contents of data_table folder:

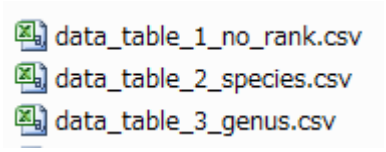
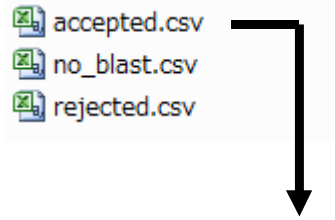



Table (which microbe has how many numbers of hits from BLAST) for rank

Contents of hits_result folder:




No_blast_hit: reads that did not satisfy filter conditions from BLAST execution

rejected_hit: reads that did not satisfy Blast result filter conditions from metagenome analysis execution

Accepted_hits: Table (which read belongs to which microbe) for each rank. Query ID is the read name and Taxon ID and microbial name will be displayed for each rank.

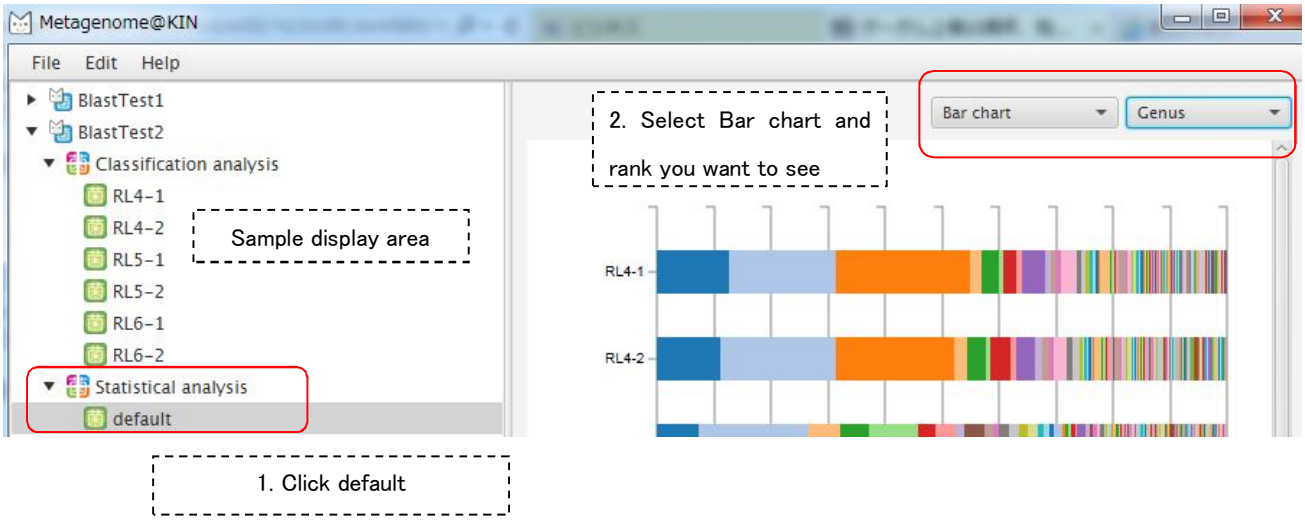
| Query ID | No_Rank | | Species | | Genus | | Family | | Order | | Class | | Phylum | | Superking | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| lcl\|HCQJIEG01CZK | 583345 | Methy | 359408 | Methyloter | 359407 | Methyloter | 32011 | Methylophi | 206350 | Methylophi | 28216 | Betaproteo | 1224 | Proteobact | 2 | Bacteria |
| lcl\|HCQJIEG01D3X | NA | NA | 881957 | Bacillus sp | 1386 | Bacillus | 186817 | Bacillaceae | 1385 | Bacillales | 91061 | Bacilli | 1239 | Firmicutes | 2 | Bacteria |
| lcl\|HCQJIEG01B3A | NA | NA | 667502 | Bacillus sp | 1386 | Bacillus | 186817 | Bacillaceae | 1385 | Bacillales | 91061 | Bacilli | 1239 | Firmicutes | 2 | Bacteria |
| lcl\|HCQJIEG01EP7 | NA | NA | 454662 | Cellvibrio s | 10 | Cellvibrio | 135621 | Pseudomor | 72274 | Pseudomor | 1236 | Gammaprot | 1224 | Proteobact | 2 | Bacteria |
| lcl\|HCQJIEG01AWC | NA | NA | 1096000 | Streptomyc | 1883 | Streptomyc | 2062 | Streptomyc | 2037 | Actinomyc | 1760 | Actinobacte | 201174 | Actinobacte | 2 | Bacteria |
| lcl\|HCQJIEG01DNT | NA | NA | 1097711 | Bacillus sp | 1386 | Bacillus | 186817 | Bacillaceae | 1385 | Bacillales | 91061 | Bacilli | 1239 | Firmicutes | 2 | Bacteria |

## VIII.Bar Graph Display from Multiple Samples

In order to analyze multiple samples (= multiple BLAST result file) at the same time, the microbial construction of the all samples can be shown in bar graphs.

Clicking “default” under “Statistical Analysis” on the sample display area, bar graphs will be displayed (selecting Bar chart from pull-down menu and proper rank)

Additionally, each cluster analysis result is also available from the pull-down menu.



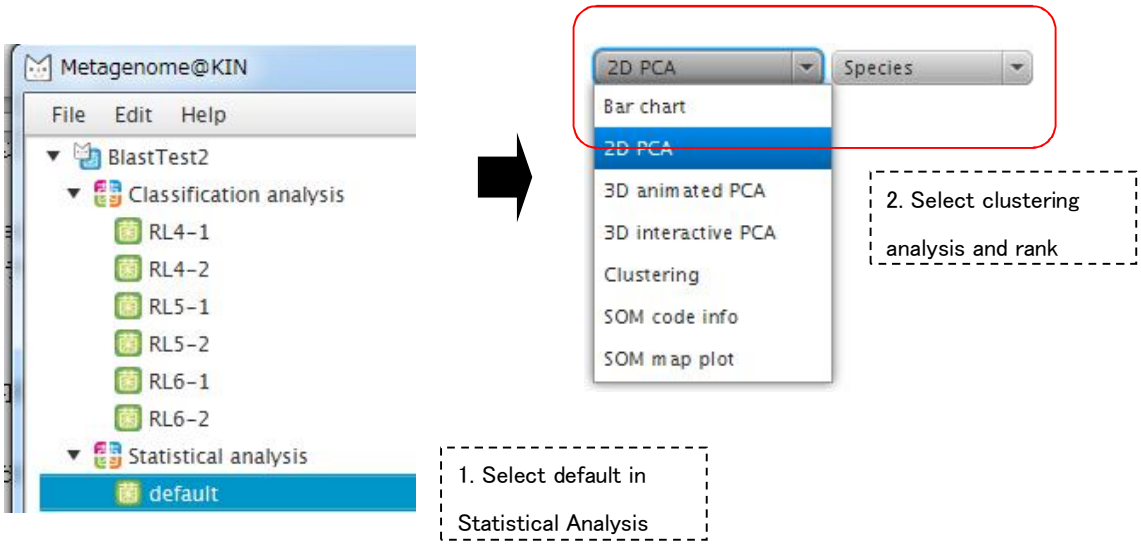
## IX.Cluster Analysis Result Display

In a case of analyzing multiple samples, Metagenome@KIN executes following 5 kinds of analyses.

- ✓ PCA 2D: Principal Component Analysis (2D)
- ✓ PCA 3D Animation: Principal Component Analysis (3D、animation)
- ✓ PCA 3D Interactive: Principal Component Analysis(3D、interactive)
- ✓ Clustering: Hierarchical clustering
- ✓ SOM: Self-organizing Maps

To view clustering analysis results, select one from the pull-down menu after selecting "default" under "Statistical Analysis" in the project folder.

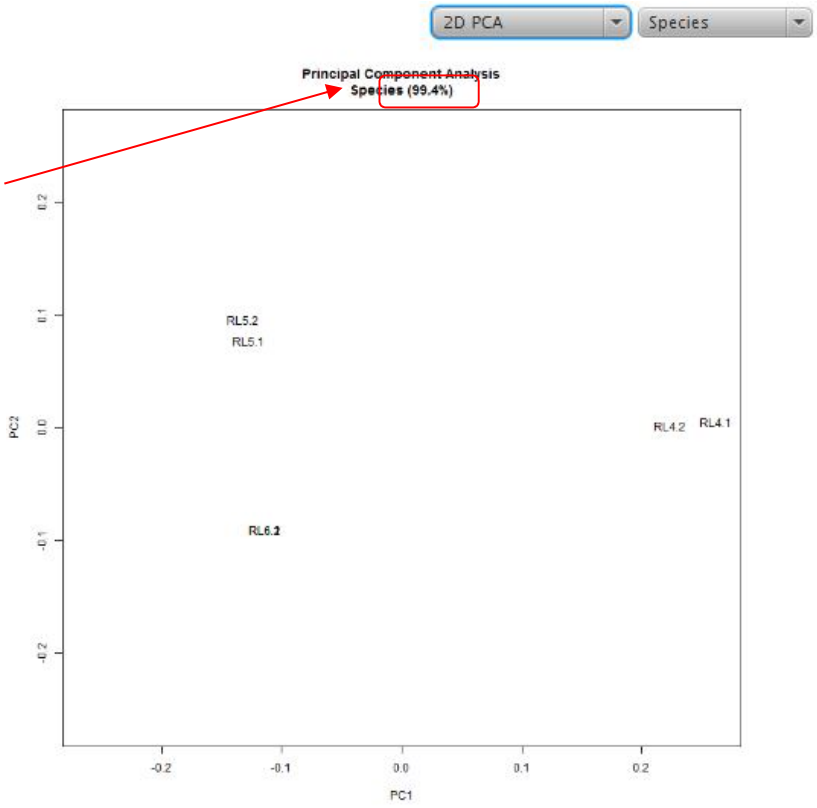
Once you select a rank from the pull-down menu, its clustering analysis will begin.



## X.2D PCA Analysis Result Display

Selecting 2D PCA, a 2D plot image for each microbe will be displayed. The % in the image indicates the information amount of PC1 and PC2 in the plot.

You can alter the rank display and export the 2D PCA analysis plot image from the Export function from File menu.

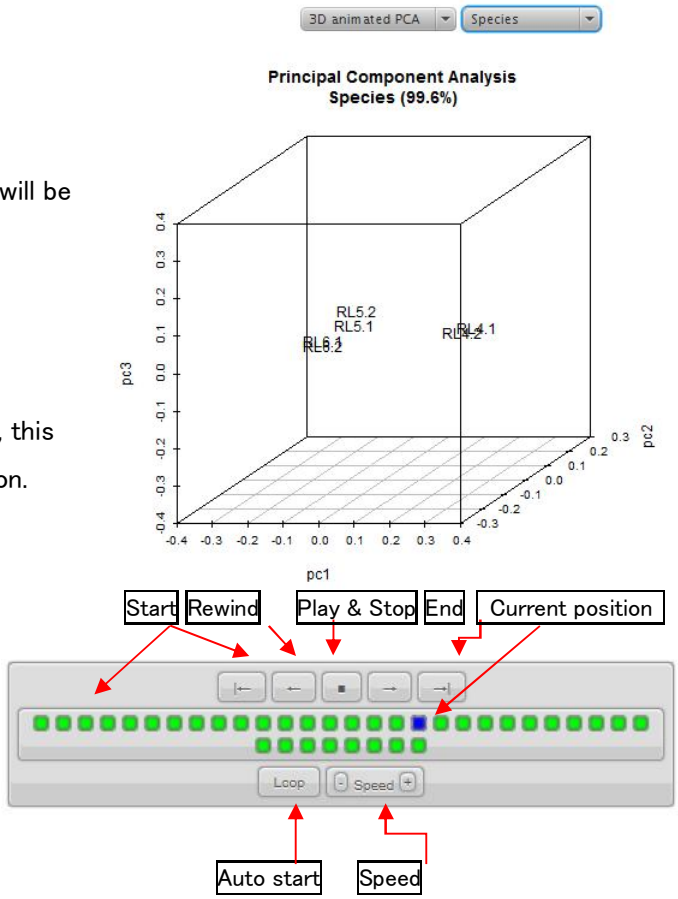


## XI.3D PCA Analysis Result Display

Selecting 3D animated PCA, a principal component analysis in 3D animation plot for each microbial level will be created.

Selecting 3D interactive PCA, a plot of principal component analysis in 3D will be displayed. However, this 3D plot does not work on Metagenome@kin application.

Instead, you can use FireFox in order to view the 3D interactive PCA by exporting the file.
Then you can modify angles and the size of the plot.

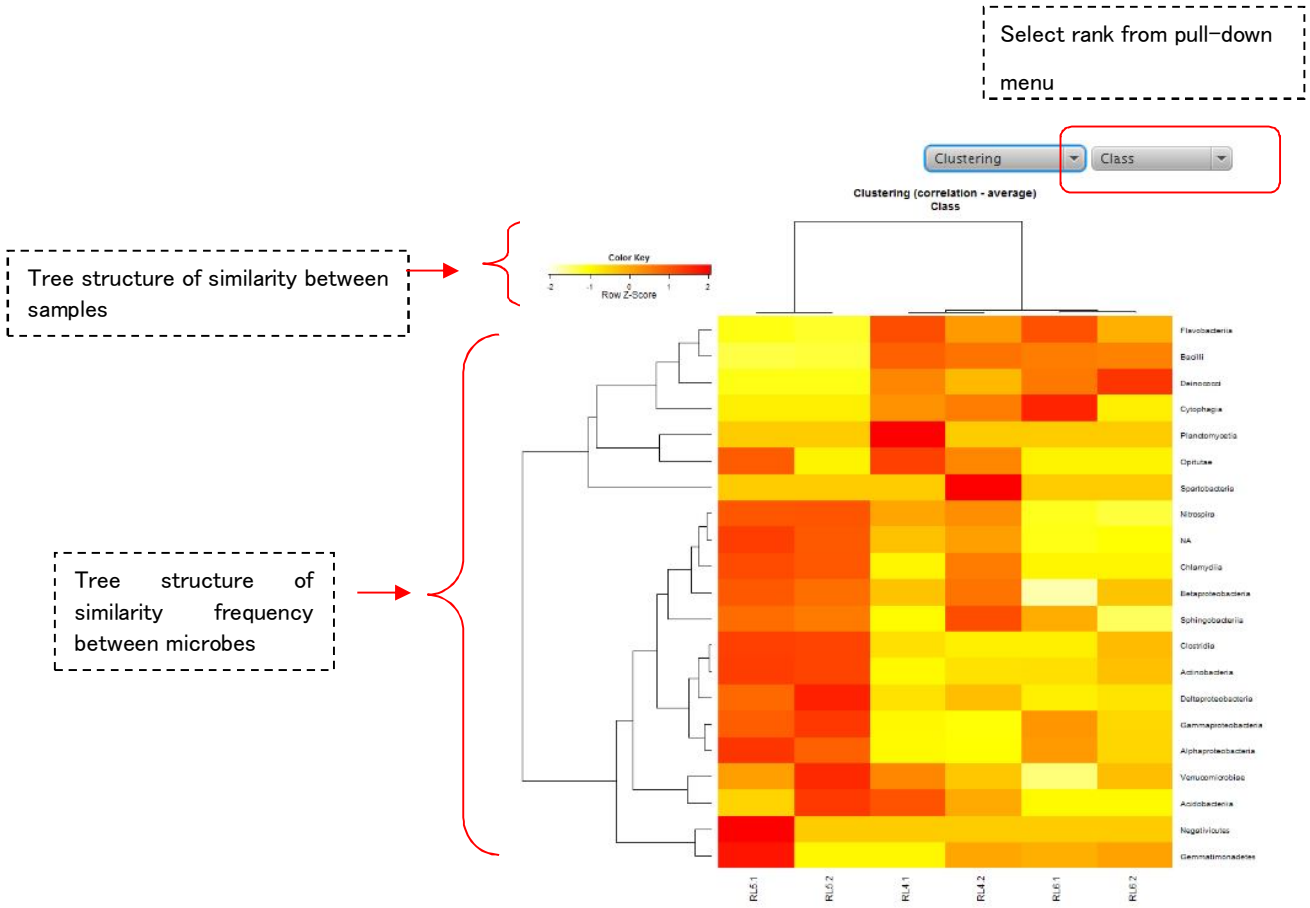



How to export 3D interactive plot:
On the menu, File –> Export Active Chart or Table.
A html file will be created and it will be available under FireFox.

## XII.Hierarchical Cluster Analysis Result Display

Hierarchical clustering result will be displayed by selecting Clustering from the pull-down menu.

Highest frequency will be colored in red and medium is yellow and lowest is white on the analyzed sample.



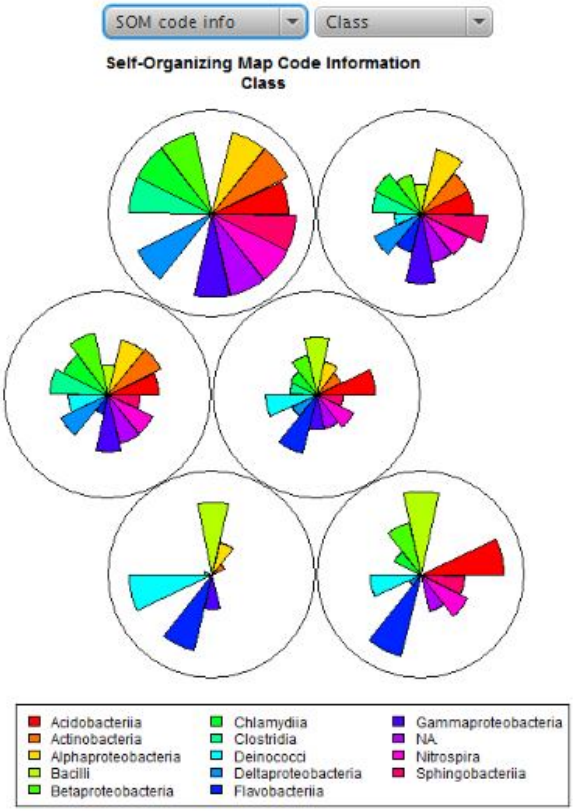

You can export the clustering analysis result image in a PNG format from the Export function from File menu.

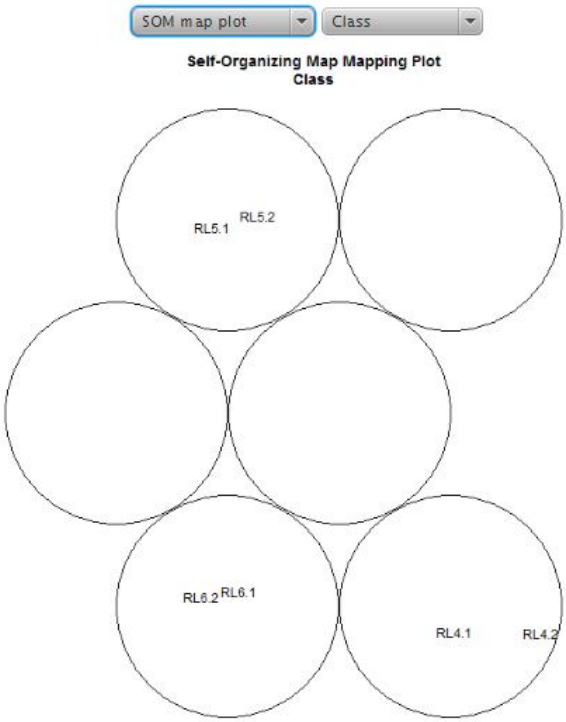
## XIII.SOM Analysis Result Display

A result of self-organizing map will be created by selecting "SOM code info" or "SOM map lot" from the pull-down menu. A SOM clustering result file consists two clustering image files (_code.png and _map.png) for each classified level. "SOM map plot" on the right shows clustering result information for samples, and "SOM code info" on the left shows microbial structural component for each rank (= pie).

On a SOM map plot, samples in the same circle have similar microbial components and there is similarity between samples in flanking circles. SOM code info concretely addresses what kind of microbe is used for similarity.

On SOM Code info, an arc color in a circle shows each microbe, and each arc's angle is the same. The difference of pie diameters correspond to a component ratio of sample microbes which construct its pie. Therefore, pies in a circle and adjacent circles have similar sizes and color. Pies in a SOM circle list top 14 microbes have big dispersion between samples (big distributions between samples).

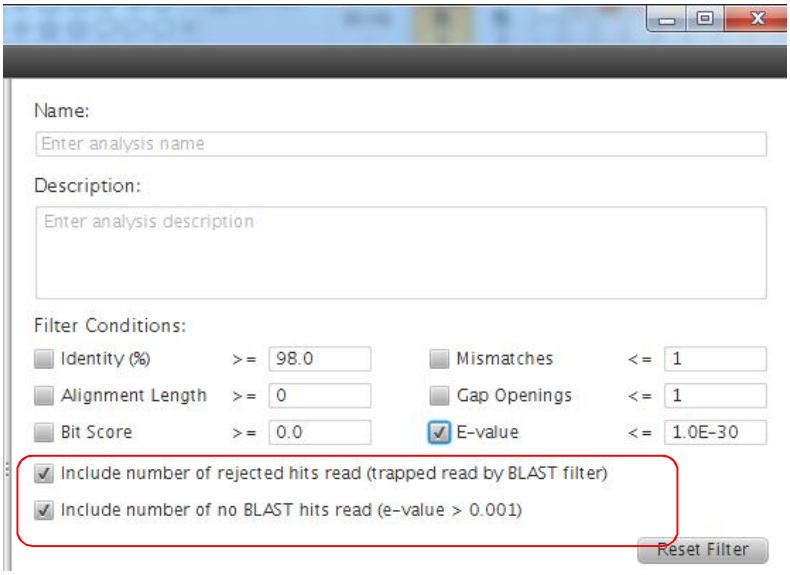



You can export a SOM analysis result image in a PNG format from the Export function in File menu.

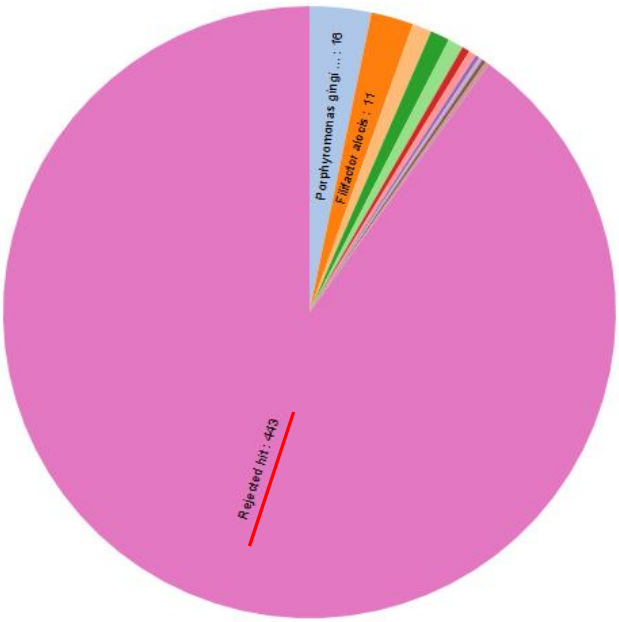
## XIV.Metagenome Analysis for Reads who Do Not Pass Blast Filter Conditions

By checking boxes of "Include number of…" on analysis execution menu, you can analyze reads which do not pass the filter conditions from BLAST results and unregistered reads in database.

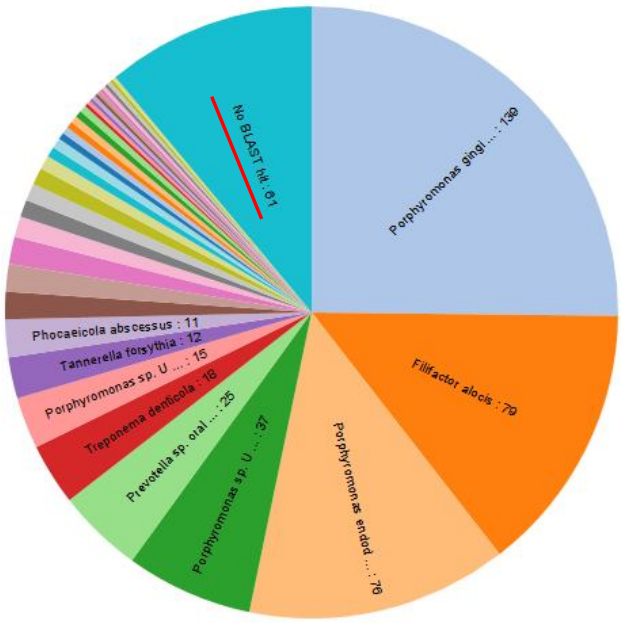



By checking "Include number of rejected hits read (trapped read by BLAST filter)", reads (as Rejected Hits) which did not pass filter conditions at KIN analysis execution can be displayed on graphs or clustering analysis. By checking "Include number of no BLAST hits read", the number of reads from microbes which do not have phasic cut-off conditions of BLAST execution will appear as No BLAST hits on graphs or clustering analysis.

A case of reject hits in analysis



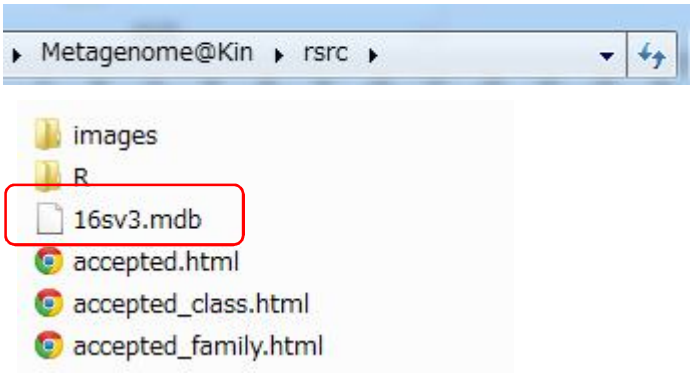

a case of no BLAST hits in analysis


No BLAST hit : 81
Porphyromonas gingi ... : 130
Phocaeicola abscessus : 11
Tannerella forsythia : 12
Porphyromonas sp. U ... : 15
Treponema denticola : 18
Prevotella sp. oral ... : 25
Porphyromonas sp. U ... : 45
Porphyromonas endod ... : 76
Filifactor alocis : 79

## XV.How to Upgrade 16SrRNA Database

Metagenome@KIN contains database of microbial names for Blast result files.

From a download site in current database file (mdb file) presented by World Fusion, you can upgrade your database by switching the .mdb file in rsrc folder within installed Metagenome@KIN folder as shown below.

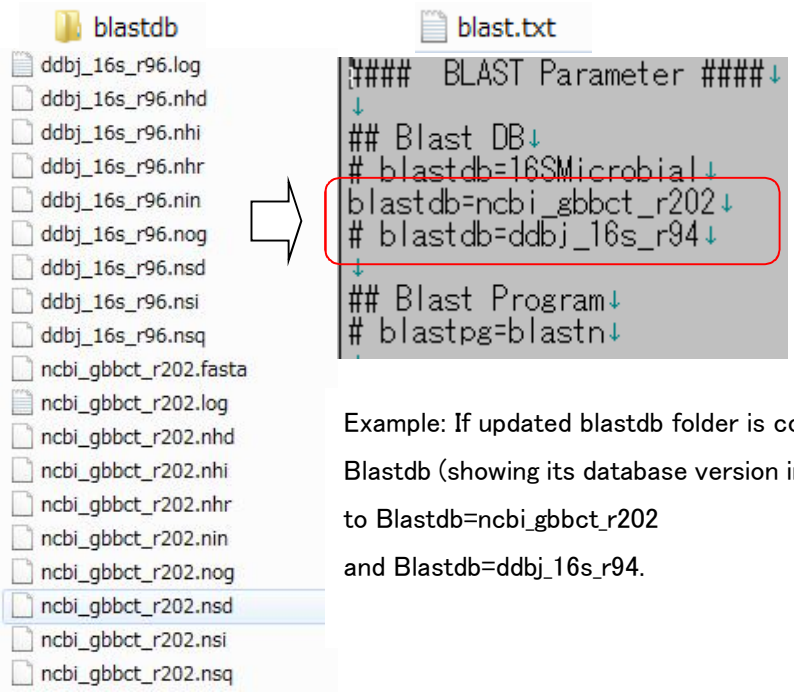



＊In case that you've obtained your BLAST result from a local Blast program.

When upgrading Metagenome@KIN database, you may need to upgrade the referred database as well for BLAST execution.

1. From a download site in current database file (mdb file) presented by World Fusion, you download and unzip a updated file for Local BLAST and switch the new "blastdb" folder from the old "blastdb" folder in the local BLAST program.

2. Open "blast.txt" in the folder where Local Blast is located. Then edit the Blast database version name in the section of "## Blast DB". The version number should match as the number in the blastdb folder.




Example: If updated blastdb folder is constructed as left

Blastdb (showing its database version in Blast.txt) needs to be changed

to Blastdb=ncbi_gbbct_r202

and Blastdb=ddbj_16s_r94.

## XVI.Reference

**Principal Component Analysis and Clustering:**

「An R and S-PLUS Comparison to Multivariate Analysis」 by Brian S. Everitt 2005 Springer.

**Self-organizing map:**

「Self-Organizing Maps」 by Teuvo Kohonen 2001 Springer

**Application of multivariable statistics for 16s RNA metagenome data**

Liu B, Faller LL, Klitgord N, Mazumdar V, Ghodsi M, et al. (2012) Deep Sequencing of the Oral Microbiome Reveals Signatures of Periodontal Disease. PLoS ONE 7(6): e37919. doi:10.1371/journal.pone.0037919

## XVII.Current Functional Limitations

＊Limits for license issuing

Metagenome@KIN is only available under an installed PC with proper physical address (MAC address) from a provide license file. If a PC does not have a network device, you cannot use the software due to unavailability of proving the physical address.

＊Limits for 3D interactive PCA graph display

Due to JavaFX specifications used in Metagenome@KIN graphic designs, you cannot control 3D interactive PCA results. In order to be able to control changes of display angles by drag & drop or sizes of the mouse wheel, you need to export the 3D interactive PCA graph in html format and see the result by FireFox.

## XVIII.How to Uninstall

Control Panel –> “Uninstall a program” under Program and select Metagenome@KIN